



Changes in snow cover alter nitrogen cycling and gaseous emissions in agricultural soils

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ABSTRACT

Climate change-related increases in winter temperatures and precipitation, as predicted for eastern Canada, may alter snow cover, with consequences for soil temperature and moisture, nitrogen cycling, and greenhouse gas fluxes. To assess the effects of snow depth in a humid temperate agricultural ecosystem, we conducted a two-year field study with (1) snow removal, (2) passive snow accumulation (via snow fence), and (3) ambient snow treatments. We measured in situ N_2O and CO_2 fluxes and belowground soil gas concentration, and conducted denitrification and potential nitrification laboratory assays, from November through May. Snow manipulation significantly affected winter N_2O dynamics. In the first winter, spring thaw N_2O fluxes in snow removal plots were 31 and 48 times greater than from ambient snow and snow accumulation plots respectively. Mid-winter soil N_2O concentration was also highest in snow removal plots. These effects may have been due to increased substrate availability due to greater soil frost, along with moderate gas diffusivities facilitating N_2O production, in snow removal plots. In the second winter, spring thaw N_2O fluxes and soil N_2O concentration were greatest for ambient snow plots. Peak fluxes in ambient snow plots were 19 and 24 times greater than in snow accumulation and snow removal plots, respectively. Greater soil moisture in ambient snow plots overwinter could have facilitated denitrification both through decreased O_2 availability and increased disruption of soil aggregates during freeze-thaw cycles. Overall, results suggest that effects of changing snow cover on N cycling and N_2O fluxes were not solely a direct effect of snow depth; rather, effects were mediated by both soil water content and temperature. Furthermore, the fact that treatments with greatest mid-winter belowground N_2O accumulation also had greatest spring thaw N_2O fluxes in both years suggests the hypothesis that high spring thaw fluxes were due not only to spring soil conditions, but also to an effect of soil conditions in frozen soils that had facilitated N_2O production throughout winter.

1. Introduction

Humans have greatly increased fluxes to the atmosphere of the potent greenhouse gas nitrous oxide (N_2O), and approximately 66% of anthropogenic fluxes are due to agricultural activities (Davidson and Kanter, 2014). These fluxes continue in winter, even when soils are frozen (Dorland and Beauchamp, 1991; Teepe et al., 2001), with winter emissions making up 45–80% of annual emissions in agricultural systems (Röver et al., 1998; Teepe et al., 2000; van Bochove et al., 2000). Emissions are often greatest during freeze-thaw cycles and at spring thaw (Goodroad and Keeney, 1984; Cates and Keeney, 1987; Nyborg et al., 1997).

Climate-related increases in annual temperature and precipitation as predicted for eastern Canada and the northeast United States could

greatly alter the amount, timing, and continuity of winter snow cover (Huntington et al., 2004; Hayhoe et al., 2006; Brooks et al., 2011). It is unclear whether changes in snow cover will increase or decrease greenhouse gas emissions throughout winter and during thaws. A meta-analysis of snow manipulation experiments found that the strength and direction of the effect of decreasing snow depth varied by latitude and climate: snow removal caused greater increases in spring N_2O flux at lower latitudes with greater annual precipitation and less variable soil temperatures (Blankinship and Hart, 2012). A few studies have employed snow removal experiments in agricultural fields. These studies found that winter N_2O production and spring thaw emissions often increased along with the greater soil frost and decreased soil O_2 in soils associated with snow removal (Maljanen et al., 2007, 2009; Yanai et al., 2011, 2014). However, whether fluxes were greater from snow removal

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plots than control plots depended on soil type (sand, mull, or peat) and the timing and extent of snow removal (Maljanen et al., 2009).

Winter soil processes and N_2O fluxes are directly affected by the timing and extent of precipitation, and establishment of a consistent seasonal snow cover (Jones, 1999; Wipf and Rixen, 2010; Brooks et al., 2011). Snow acts as an insulator, eventually decoupling soil temperatures from those of the air, and moderating the depth and extent of winter soil frost and the occurrence of freeze-thaw events (Sharratt et al., 1992). Soil frost, in turn, affects gaseous diffusion, the availability of water, and transport of substrates, and ice formation disrupts organic compounds and microbial cells, increasing available soil organic carbon (C) and nitrogen (N) (Christensen and Tiedje, 1990; Christensen and Christensen, 1991; Brooks et al., 2011). This could increase N_2O fluxes, particularly during subsequent thawing (Maljanen et al., 2007, 2009; Yanai et al., 2011, 2014). Snow depth also affects the infiltration of water into soil during mid-winter and spring thaws, and contributes to soil N availability through addition of inorganic N contained in the snow (van Bochove et al., 2000).

Denitrification, the anaerobic, microbially-mediated reduction of NO_3^- to N_2 , has been demonstrated to be the main source of N_2O in laboratory and field experiments with frozen and thawing soils (Öquist et al., 2004; Mørkved et al., 2006; Wagner-Riddle et al., 2008). The rates and end products of denitrification (i.e., N_2O vs. N_2) are controlled by aspects of the soil environment that are affected by snow depth, such as soil temperature, moisture, and frost. Low temperatures may favor N_2O production due to low functioning of N_2O reductase at temperatures below $\sim 5^\circ\text{C}$ (Sahrawat and Keeney, 1986; Holtan-Hartwig et al., 2002; Dörsch and Bakken, 2004), although the effect of low temperatures on N_2O production versus reduction has been shown to vary by soil source, presumably due to variation in microbial communities (Dörsch and Bakken, 2004). The restriction of gas diffusion by soil frost can directly promote denitrification by decreasing O_2 availability. However, restricted gas diffusion and low O_2 availability could also indirectly limit N_2O production, via two routes. First, evidence from unfrozen soils indicates that a lack of O_2 limits the production of NO_3^- by aerobic nitrification, suggesting that denitrification could become NO_3^- -limited (Firestone et al., 1980). Second, N_2O is more likely to be reduced to N_2 when its diffusion out of the soil is limited (Sahrawat and Keeney, 1986; Davidson et al., 2000). Therefore, greatest production of N_2O may occur at moderate gas diffusivities (e.g. if frost layers are permeable or discontinuous, or at field capacity for thawed soils), when oxic zones and anoxic microsites exist, facilitating both denitrification and nitrification (Davidson et al., 2000). However, it is unclear how changes in soil temperature, frost, and moisture in response to altered snow depth will interact to affect N_2O production over winter and during thawing.

To assess the effects of snow depth on soil N cycling and gaseous emissions in humid temperate agricultural ecosystems, we conducted a two-year field study with snow removal, passive snow accumulation, and ambient snow treatments. We hypothesized that soil temperature and moisture, and frost intensity and duration, would differ between soil removal, ambient snow, or snow accumulation treatments, resulting in differences in the pattern and intensity of N_2O fluxes.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted over two years at the Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada. Fields were in a potato (*Solanum tuberosum*)-barley (*Hordeum vulgare*) rotation. In each winter, the study was conducted in a field following the harvest of the potato crop, so that any potential crop effects would be consistent. To achieve this, two different, adjacent plot areas were used in the two winters.

Fredericton, NB, has an average winter (November through April)

temperature of -3.0°C and receives an average of 215 cm snow per year, with winter monthly average snow depths from 1 to 17 cm (1981–2010, Fredericton CDA station, Environment Canada). However, variability in snow depth can be substantial; the greatest recorded snow depth at this station was 112 cm in March 1963. Soils at this site are Orthic Humo-Ferric Podzols according to the Canadian system of soil classification. Soil properties were determined in the surface soil (0–7.5 cm) from three samples of each treatment from each year's plot area. Soil pH was 6.0 and consistent across plot areas. Soil textures varied between the two adjacent plot areas. In the first year, soil texture was a loam with 45% sand and 12% clay, as determined by the pipette method. In the second year, soil had significantly greater sand and significantly lower silt and clay content; soil texture was a sandy loam with 68% sand and 6.6% clay. Total soil C and N, as determined by the dry combustion method, were significantly greater in the field used in the first year than in the second, but did not differ significantly by treatment. In the first year, soil N and C contents were $1.6 \pm 0.04 \text{ g kg}^{-1} \text{ N}$ and $16.7 \pm 0.5 \text{ g kg}^{-1} \text{ C}$ respectively, and in the second year, soils were $1.2 \pm 0.09 \text{ g kg}^{-1} \text{ N}$ and $10.3 \pm 0.09 \text{ g kg}^{-1} \text{ C}$ respectively.

Experimental manipulations consisted of snow removal, snow accumulation, and ambient snow treatments. Due to the logistics involved in removing or accumulating snow, a random block design could not be used, and replicate plots were located within larger treatment areas. In snow removal plots, snow was removed when it accumulated to greater than 10 cm depth. Removal typically occurred within 48 h after snow had ceased falling, but this time period was occasionally increased due to consecutive heavy snowstorms. Snow was removed with shovels, or with a snow blower when soils were frozen. A thin (1–4 cm) layer of snow was left to maintain albedo. In snow accumulation plots, snow was passively accumulated using a snow fence (Home Depot Model #3407). The snow fence was oriented perpendicular to the northeast, the predominant wind direction during snowfall. Replicate plots were located along the peak of the snow drift, in a line parallel to the snow fence, approximately 4 m downwind from the fence. Due to space constraints related to the length and orientation of the snow fence, three replicate plots were used for the snow addition treatment while four replicate plots were used for the snow removal and ambient treatments. Measurements of soil analytes, N cycling processes (denitrification and nitrification rates), and gas concentrations were done using three replicates per treatment, whereas gas fluxes were measured in three replicates plots in the snow accumulation treatment and four replicate plots in snow removal and ambient treatments.

All treatment areas and replicate plot sizes were decreased the second year due to space restrictions to avoid wetter parts of the field where potatoes had grown poorly the previous growing season. The sizes of full treatment areas were as follows: in winter 2013–2014, snow removal: 14 m \times 44 m; snow addition: 30 m \times 52 m; ambient snow: 20 m \times 30 m; in winter 2014–2015, snow removal: 12 m \times 25 m; snow addition: 30 m \times 43 m; ambient snow: 14 m \times 34 m. Replicate plots for gas flux measurements, located within each treatment area, were 4 m \times 6 m the first year and 3 m \times 6 m or 4 m \times 4 m the second year. Replicate plots for soil sampling, also located within each treatment area, were a minimum of 6 m \times 7 m the first year and 4 m \times 4 m the second year.

2.2. Environmental measurements

Snow depth was measured manually with a meter stick at each gas flux collar on each gas sampling date in both winters. In the second winter, snow depth was also measured at hourly intervals using a snow sensor (SR50AT with CR200X data logger, Campbell Scientific) installed in each of the three treatment plots.

Soil temperature and volumetric (liquid) water content were monitored at one hour intervals at 5 cm depth from November through May using one Decagon EM50 data logger equipped with 5TE sensors in

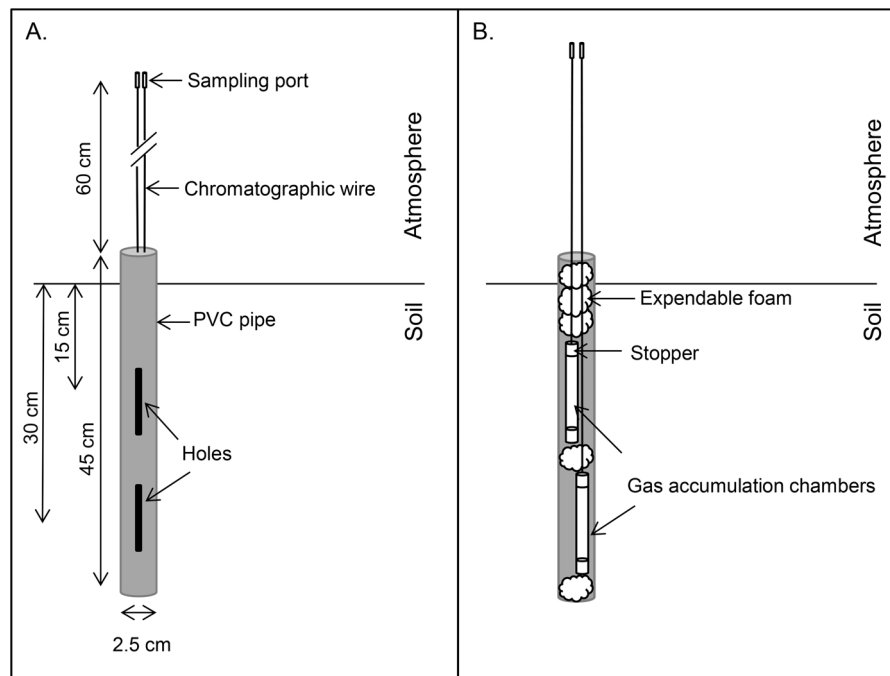


Fig. 1. Soil gas wells. See materials and methods for details on construction.

each of the three treatment plots. Two sensors were placed at the 5 cm depth, and presented values are means. Data were not recorded for the snow removal plot in the first winter, as sensors were accidentally disconnected in December.

2.3. Soil gas accumulation and emissions

Gas sampling was conducted approximately bi-weekly from November through May, with more frequent weekly sampling during spring thaw to observe anticipated declines in soil gas and peaks in greenhouse gas emissions. In total, there were 16 gas sampling dates in the first winter and 17 in the second. Soil gas accumulation was measured using static soil gas wells inserted into the soil at three replicate plots for each treatment. Gas wells were modified from [Burton \(2013\)](#) (Fig. 1). Gas wells consisted of a polyvinyl chloride (PVC) pipe of 2.5 cm in diameter with two oblong holes that were cut along the PVC pipe to allow gas exchange. Two gas accumulation chambers were inserted inside the PVC pipe and centered at 15 and 30 cm depth. The PVC pipe was sealed at each end and between accumulation chambers with expandable foam. When inserted in soil, the gas accumulation chambers equilibrate with ambient soil gases but prevent water from entering the chamber. To construct the gas accumulation chambers, platinum-cured silicone tubing (Cole-Parmer 95802-22, 1/2" inner diameter, 11/16" outer diameter) was stoppered at both ends with rubber stoppers (Fisher Scientific 03-225-5). Stainless steel tubing (1/16" outer diameter x 0.030" inner diameter; Chromatographic Specialties C49305) was inserted into the top of each gas accumulation chamber and run along the inside of the PVC pipe to the top of the gas well, where sampling ports were created with Tygon tubing and female Luer hose barb adapters (1/16" inner diameter female Luer thread barb; Cole-Parmer 45500-00) (Fig. 1). In the first year, sampling ports were located approximately 15 cm above the ground; however, ports were difficult to access in deep snow, and resulted in missing data for snow accumulation plots in early April 2014. For this reason, wires were extended approximately a meter to allow for access to sampling ports in deep snow in winter 2014–2015. Gas samples (10 mL) were collected using a luer-lok syringe fitted with a stopcock, and transferred to 5.9 mL glass vials (Exetainer, Labco Limited). Gas samples were analyzed for N₂O and CO₂ concentration using a gas chromatograph (Model 3800,

Varian, Walnut Creek, CA) fitted with an electron capture detector to measure N₂O, a thermal conductivity detector to measure CO₂, and a Combi-PAL Autosampler (CTC Analytics, Zwingen, Switzerland). Because trends and significant effects (treatment and sampling date) were similar for soil N₂O and CO₂ concentrations at 15 and 30 cm depth, the results of gas concentrations at 15 cm depth was presented herein while the results of 30 cm depth is included as Supplemental figure.

Soil or snow surface N₂O and CO₂ emissions were measured using static chambers. In the fall, base collars (polyvinyl chloride (PVC) tubes, 20 cm diameter, 20 cm length), were inserted into the soil in the center of each gas flux plot with approximately 10 cm remaining above the surface. Collars were painted white to minimize heat absorption during measurements. To allow for sampling throughout the winter with minimal disturbance of the snow pack, collars were maintained at a height above the surface of the snow by stacking and removing 10 cm pipe sections, secured with wrapping plastic and packing tape to form a tight seal ([Chantigny et al., 2017](#)). Collars were usually added 1–3 days before predicted snowfall. On each sampling date, sampling typically began between 8 and 10 am and was conducted first in snow accumulation plots and then in both snow removal and ambient snow plots. Vented, insulated PVC chambers, covered in reflective foil and with closed cell foam on their bottom edges, were placed on the base collars. Samples (20 mL) were collected through a rubber septum using a 20 mL plastic syringe at 0, 20, 40, and 60 min after chamber deployment and then transferred to pre-evacuated, 12 mL glass vials (Exetainers, Labco Limited). Samples were analyzed with a gas chromatograph as described above.

Gas fluxes were calculated as linear accumulation of gases over time, as accumulation was consistently linear. Chamber volumes, including collar extensions, were corrected by subtracting the approximate water volume of snow in the collars as follows. On each sampling date, the height of snow in each collar was assessed visually relative to the top of the collar and extensions. Snow depth was converted to snow volume and then total water volume using a representative snow density for that sampling date. To determine snow density, snow pits were dug to the frozen ground in an ambient patch of snow separate from treatment plots, and a representative sample was taken from each distinct layer of snow or ice along the depth gradient. Snow samples were taken using a 10.8-cm long aluminum core, 6.35 cm in diameter,

inserted into the snow horizontally. It was not possible to use the aluminum core when an ice layer was found in the snow profile; in that case, ice samples were cut into rectangular prisms and measured. Samples were placed in pre-weighed plastic bags and weighed upon returning to the lab. Total representative snow density was determined by multiplying the density of each layer by its proportion of the total height.

Chantigny et al. (2017) used a similar winter chamber method to measure surface emissions of greenhouse gases. However, whereas our gas flux calculations accounted for the gas volume in the snow within the collars as well as the gas volume above the snow, calculations by Chantigny et al. accounted for only the headspace above the snow. To determine whether this difference in calculation method would affect conclusions, we compared fluxes as calculated above to those calculated following Chantigny et al. (2017) and found no difference in statistical significance among treatments or sampling dates.

2.4. Soil sampling

Soils were sampled approximately monthly, with biweekly samples during two periods: after initial snow cover, to ensure sufficient samples under snow, and during spring thaw, to better represent this period of active biogeochemical change. In total, soils were sampled ten times each winter. On all sampling dates, soil temperatures at 7.5 cm depth were measured in each replicate plot using a thermometer. Soil sampling procedure varied depending on whether soils were frozen. When soils were not frozen, a hand trowel was used to collect four 250 mL subsamples from each replicate plot, and subsamples were combined into one sample in a plastic container. When soils were frozen, surface soil was broken up with a generator-powered rotary hammer (SDS-max Combination Hammer, Robert Bosch Tool Corporation) with a chisel bit, and a total of 1 L of soil was collected, further broken up with a mallet, and mixed. In each case, the top 7.5 cm of soil was collected. When snow was present, it was removed with a shovel, and when surface ice was observed in late winter 2013–2014, it was removed with a shovel or chisel. Ice integrated into the soil was not removed.

For each sample, 250 mL of soil was aliquoted into each of two pre-weighed 1 L jars to be held in preparation for the denitrification assay. This corresponded to approximately 233 ± 90 g wet soil and 163 ± 4 g dry soil (mean \pm s.e. for all treatments and sampling date). The jars were kept frozen during sampling. Once in the laboratory, the samples were immediately placed in incubators set at the measured temperatures in the field. The remaining soil was also kept frozen on snow until all samples were brought back to the laboratory, where aliquots were weighed for the potential nitrification assay and soil analyte extraction, and soil jars were weighed before beginning the denitrification assay.

2.5. Denitrification and N_2O and CO_2 flux assay

Two different sets of 1 L jars (250 mL soil) were used to measure denitrification rate and N_2O emissions. Denitrification was assayed with the acetylene block method (Groffman et al., 1999). One set of jars was incubated with the addition of 10% (vol/vol) acetylene (C_2H_2 ; inhibits the reduction of N_2O to N_2) to quantify total denitrification. The second set of jars was incubated with the addition of 10% compressed air to quantify total N_2O and CO_2 emissions. There were three replicate jars per snow manipulation treatment, i.e. one jar from each field plot replicate. Jars were tightly closed and incubated in a low temperature incubator (Panasonic MIR-154-PA) at the mean soil temperature measured in the field for each treatment (Table S1 and S2). Gas samples (20 mL) were collected from the jar headspace with a syringe through a rubber septum after 1, 4, 8, and 20 h of incubation, and transferred to 12 mL pre-evacuated vials (Exetainers, Labco Limited). Samples were analyzed using a gas chromatograph as described above. Due to non-linear accumulation of gases, particularly at higher rates, emission rates

were calculated as follows. A logarithmic curve was fit to the data, implying a changing rate of production over time. The production rate reported here was the instantaneous rate of production at the first sampling time point (1 h). In calculations of N_2O or CO_2 produced or present at each time point, we took into account the mass of gases removed at previous sampling time points as well as CO_2 and N_2O dissolved in soil solution. Dissolved CO_2 was calculated using the `aquaenv` function in the R package `AquaEnv` (Hofmann et al., 2010), taking temperature and soil pH into account to calculate total carbonate concentration. Dissolved N_2O was calculated following the equations presented in the errata of Roper et al. (2013). As soil moisture was not adjusted, so as to avoid physical or salinity-related disturbance, we accounted for potentially variable soil moisture in calculations of rates per mass of dry soil.

Replicates for the snow removal treatment on January 24 were lost due to incubator malfunction. For balance, this date was removed from statistical analysis.

2.6. Potential nitrification

Potential nitrification assays were conducted at room temperature (approx. 20 °C) under oxic and non-limiting N substrate conditions using a soil slurry method modified from Hart and Stark (1997) and Wertz et al. (2011). Experiments were conducted shortly after return to the laboratory, once soil samples had been weighed. To remove initial soil nitrate, field moist samples (2 g) were shaken in potassium phosphate buffer (1 mM, pH 7.2) for 15 min, then centrifuged for 5 min at 4000 rpm ($3023 \times g$). The supernatant was discarded and samples were resuspended in 20 mL of the same potassium phosphate buffer with addition of NH_4Cl (1.44 mM $N-NH_4$; $200 \mu g N g^{-1}$ soil). Slurries were transferred to 125 mL Erlenmeyer flasks, covered with Parafilm, and shaken at room temperature. Aliquots (1.8 mL) were collected at the start of the incubation and 2–3 times over 24 to 48 h; only time points showing linear production of NO_3^- were included in the final analysis. Aliquots were centrifuged for two min at 10,000 rpm ($10,621 \times g$), and supernatants were filtered (0.45 μm) and stored at $-20^\circ C$ until further analysis. Nitrate concentrations were determined using Szechrome NAS reagent (Polysciences, Inc., Warrington, PA), and potential nitrification activity was considered to be the production of nitrate over time (Wertz et al., 2012).

2.7. Soil analytes and moisture

Soil nutrients and organic carbon were extracted by shaking 25 g field moist soil with 50 mL 0.5 M K_2SO_4 for one hour at room temperature (approx. 20 °C). Extracts were filtered using vacuum filtration, then stored at $-20^\circ C$ until analysis. Concentrations of soil nitrate (NO_3^-), ammonium (NH_4^+), and extractable organic carbon (EOC) in extracts were measured using a Technicon AutoAnalyzer II system, following Technicon Industrial Methods #100–70 W, #98–70 W, and #455–76 W respectively (Technicon Industrial Systems, Terrytown, MA).

Soil moisture (gravimetric water content of collected experimental soils) was determined in soils from all replicate jars that had received compressed air in the denitrification assay. Jars were weighed when empty (before sampling) and again with freshly collected soil, before the denitrification assay. After completion of the assay, jars were placed in the oven at 105 °C for 3 days (until fully dry), placed in a desiccator to cool, and re-weighed.

2.8. Data and statistics

All analyses were conducted using R version 3.2.3 (R Core Team, 2016). Two-way ANOVAs with type III sums of squares (SS) with treatments and sampling dates as main factors were used for in situ gas fluxes, soil gas wells, denitrification, nitrification, and soil analytes.

Gravimetric water content was assessed with a three-way ANOVA with type III SS with period (winter vs. thaw), treatment, and sampling year as main factors. Residuals were examined to confirm that model assumptions, i.e. normality and homogeneity, were not violated. Data were log-transformed when necessary for normality and homogeneity of residuals. Any large residuals were evaluated and dealt with appropriately. When interactions were significant, pairwise comparisons were examined for within-date differences using Tukey's test via the *lsmeans* and *cld* functions in the R package *lsmeans* (Lenth, 2016).

Cumulative emissions throughout the non-growing season, and in the winter versus spring thaw periods, were calculated by linear interpolation between sampling dates and then summation. Cumulative emissions were considered over the full range of sampling dates, i.e., 11 November 2013 until 8 May 2014 for the first winter (170 days), and 21 November 2014 until 15 May 2015 for the second winter (176 days). The transition between winter and spring thaw periods was considered to be halfway between the sampling date where thawing was noted and the previous sampling date, at which point soils were still frozen. Spring thaw was thus determined to start on 11 April 2014 and 6 April 2015, resulting in 126 and 127 total winter days and 44 and 49 total spring days for the first and second winters respectively. Cumulative emissions were compared within winters using a two-way ANOVA with treatment and period (i.e., winter or spring thaw) as factors. When interactions were significant, pairwise comparisons among all treatments and both periods were examined using Tukey's test via the *lsmeans* and *cld* functions in the R package *lsmeans* (Lenth, 2016).

Pearson's correlations were used to assess relationships between different measurements, notably: soil NO_3^- and NH_4^+ concentrations; soil gas N_2O and CO_2 concentrations; and greenhouse gases (soil gas N_2O and CO_2 or N_2O and CO_2 fluxes) and winter conditions (snow depth, soil temperature, VWC) and soil analytes (NO_3^- , NH_4^+ and EOC). All other relationships between combinations of soil analytes, gases, and conditions were also assessed. For all correlations, results were not reported when correlations were nonsignificant or had very low r^2 values. We further assessed whether the relationship between soil N_2O and soil CO_2 concentrations was dependent on treatment using analysis of covariance (ANCOVA), with CO_2 concentration and treatment as factors, using the *gls* function of the R package *nlme* (Pinheiro et al., 2016). ANCOVA is a general linear model that combines regression and ANOVA, effectively assessing whether a correlation (i.e., between N_2O and CO_2 concentration) varies among levels of a categorical independent variable (treatment). It can also be described as comparing means of a dependent variable (N_2O concentration) across an independent variable (treatment) while controlling for a covariate (CO_2 concentration). For all analyses, statistical significance was assumed for $p < 0.05$.

3. Results

3.1. Environmental measurements

In both years, winter was marked by several periods with distinct patterns of snow depth, soil temperature, and soil water content. In the first winter, there was no snow in November and early December, and fluctuations in soil temperature at 5 cm and soil volumetric water content during that period were indicative of freeze-thaw cycles (Fig. 2a–c). In late December and early January, snow pack stabilized at greater than 20 cm depth, which maintained soil temperatures under snow at around -1°C and volumetric (liquid) water content at about 0.05 (ambient snow) to 0.1 (snow accumulation) $\text{cm}^3\text{cm}^{-3}$; in snow removal plots, soil reached -6°C . A mid-January thaw caused a spike in water content and more variable freezing temperatures in the ambient snow plot due to decreased snow depth, whereas soil temperatures in the snow accumulation plot remained close to -1°C . Late February snowfall stabilized soil temperatures in snow-covered plots at approximately -1°C until spring thaw, while soil in snow removal

plots continued to freeze to colder temperatures throughout the winter (minimum measurement: -4.3°C). Snow depth in snow accumulation plots was approximately twice that in ambient snow plots throughout the winter. From approximately late February through spring thaw in April, there were several-centimeter thick layers of ice in the snow, as well as on top of the soil and below the snow pack, in most ambient snow and snow accumulation plots, whereas the snow removal plot had more discontinuous patches of ice. During spring thaw, soils reached mean daily temperatures above 1°C in snow removal plots (mud present on April 1), followed by ambient snow (April 14) and then snow accumulation plots (April 19).

The second winter had a similar pattern of early winter variability and later winter stability (Fig. 2d–f). In December, before freezing, volumetric water content was considerably greater in ambient snow plots than other treatments. Until late January, snowfall was associated with more southerly winds, and snow was often blown away from the experimental site, causing soil in all plots to experience freeze-thaw cycles. In mid-January, a period of snow prevented soils in ambient snow and snow accumulation plots from reaching the freezing temperatures measured in snow removal plots. Snow depth increased substantially in late January in snow accumulation (94 cm) and ambient snow (37 cm) plots and was present throughout winter, stabilizing soil temperatures, while soil in snow removal plots reached colder temperatures (minimum: -10.8°C). In early February, snow depth was greater in snow accumulation plots (75 cm) than ambient snow plots (49 cm), but depths were comparable between the two treatments (~ 57 cm) by March, likely due to settling of snow with initially lower density behind the snow fence in the snow accumulation treatment. Spring thaw occurred in April, with average daily soil temperatures (5 cm depth) warming above 1°C first in snow removal (April 3), then snow accumulation (April 11), and lastly ambient snow plots (April 16).

3.2. Soil gas concentrations

Treatment and sampling date effects on soil N_2O and CO_2 concentrations at 15 and 30 cm depth measured using gas wells were similar in terms of significant effects and overall trends (Fig. 3, Fig. S1). Below, we describe results for gas concentrations at 15 cm depth, while statistics for the deeper, 30 cm gas concentrations can be found in the supplemental materials (Fig. S1).

In both years, soil N_2O concentrations varied significantly with both treatment and sampling date (Fig. 3a, b, $p < 0.001$). Concentrations were low in late fall and early winter, and began to increase in January or early February in both winters. Soil N_2O concentrations ranged from 11.1 to 60.5 $\mu\text{L L}^{-1}$ in snow accumulation plots, 4.2–44.1 $\mu\text{L L}^{-1}$ in ambient snow plots, and 8.8–127.2 $\mu\text{L L}^{-1}$ in snow removal plots between January and March in the first winter (treatment means by sampling date). N_2O concentrations also increased between January and March in the second winter, with concentrations ranging from 1.6 to 45.4 $\mu\text{L L}^{-1}$ in snow accumulation plots, 6.8–88.0 $\mu\text{L L}^{-1}$ in ambient snow plots, and 1.9–30.3 $\mu\text{L L}^{-1}$ in snow removal plots. Soil N_2O concentrations peaked in April in both winters. In the first winter, the greatest concentrations reached $216 \pm 11.9 \mu\text{L L}^{-1}$ in snow removal plots, compared to $66.4 \pm 31.2 \mu\text{L L}^{-1}$ in snow accumulation plots and $89.6 \mu\text{L L}^{-1}$ in ambient snow plots, while in the second winter, the greatest concentrations reached $110.5 \pm 42.7 \mu\text{L L}^{-1}$ in ambient snow plots compared to $66.2 \pm 19.0 \mu\text{L L}^{-1}$ in snow accumulation plots and $34.0 \pm 19.0 \mu\text{L L}^{-1}$ in snow removal plots. Soil N_2O concentrations declined to negligible values thereafter (Fig. 3). Results from the two-way ANOVA indicated that in the first winter, soil N_2O concentrations were significantly greater in the snow removal plots than in other treatments (Fig. 3a, $p < 0.001$). In the second winter, N_2O concentrations were significantly greater in ambient snow plots than in other treatments (Fig. 3b, $p < 0.001$). Soil N_2O reached greater maximum concentrations in the first winter than in the second.

Soil CO_2 concentrations similarly increased over winter in all

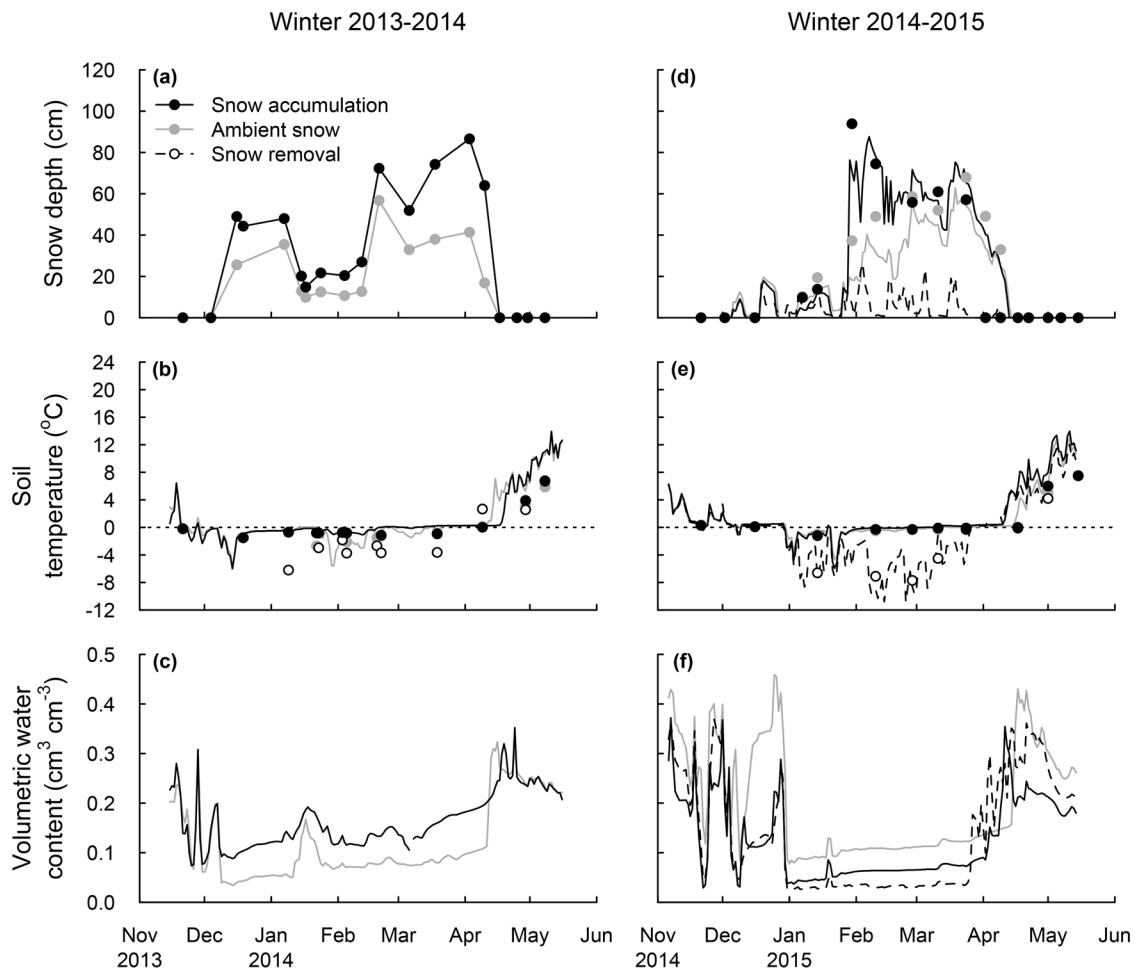


Fig. 2. Snow depth (a, d), soil temperature (b, e), and volumetric (liquid) water content (c, f) for winter 1 (a–c) and winter 2 (d–f). For snow depth, points represent measurements taken with a meterstick, whereas lines in winter 2 (d) indicate daily means of hourly measurements from snow sensors. For soil temperature and volumetric water content, lines represent daily means of hourly sensor measurements at 5 cm depth, averaged between two sensors. Points in soil temperature plots (b, e) represent measurements taken at 7.5 cm depth with a thermometer.

treatments, in both winters (Fig. 3c, d). Soil CO₂ concentrations ranged from 5.5 to $17.4 \times 10^3 \mu\text{L L}^{-1}$ in snow accumulation plots, 3.9 to $17.3 \times 10^3 \mu\text{L L}^{-1}$ in ambient snow plots, and 3.3 to $16.2 \times 10^3 \mu\text{L L}^{-1}$ in snow removal plots between January and March in the first winter (treatment means by sampling date). N₂O concentrations also increased between January and March in the second winter, with concentrations from 1.7 to $18.2 \times 10^3 \mu\text{L L}^{-1}$ in snow accumulation plots, 4.1 to $10.6 \times 10^3 \mu\text{L L}^{-1}$ in ambient snow plots, and 2.1 to $10.7 \times 10^3 \mu\text{L L}^{-1}$ in snow removal plots. Soil CO₂ concentrations peaked in April and declined over spring thaw (Fig. 3c, d). In the first year, the sampling date and the sampling date by treatment interaction were significant (Fig. 3c). In the second year, soil CO₂ varied significantly by sampling date ($p < 0.001$), with a peak in early April, and concentrations were significantly lower in snow removal plots than in other treatments ($p = 0.008$). In both years, soil N₂O concentrations were significantly positively correlated with soil CO₂ concentrations, with significant interactions between treatment and soil CO₂, as demonstrated by differences in regression slope for different treatments (ANCOVA, $p < 0.05$, Fig. S2). Specifically, slopes were relatively greater for snow removal plots in the first winter and for ambient snow plots in the second winter, such that for any given increase in soil N₂O concentration, the corresponding increase in CO₂ concentration was relatively greater for these treatments.

3.3. Soil gas emissions

In both years, in situ soil or snow surface N₂O fluxes were low to moderate in fall and early winter, with non-significant trends in December and January towards greater emissions from snow accumulation plots in the first winter, and from ambient snow plots in the second winter (Fig. 4a, b). Between November and January, N₂O fluxes ranged from 0.4 to $15.3 \text{ g N ha}^{-1} \text{ d}^{-1}$ in snow accumulation plots, 0.6 to $7.1 \text{ g N ha}^{-1} \text{ d}^{-1}$ in ambient snow plots, and 0.2 to $7.7 \text{ g N ha}^{-1} \text{ d}^{-1}$ in snow removal plots in the first winter, and 0.3 to $3.5 \text{ g N ha}^{-1} \text{ d}^{-1}$ in snow accumulation plots, 1.3 to $18.8 \text{ g N ha}^{-1} \text{ d}^{-1}$ in ambient snow plots, and 1.0 – $2.6 \text{ g N ha}^{-1} \text{ d}^{-1}$ in snow removal plots in the second winter. N₂O fluxes were typically very low to negligible during mid-winter (February and March). A double peak pattern was observed during spring thaw. The first peak occurred in early- or mid-April, with N₂O fluxes reaching rates of $4.1 \pm 2.2 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $2.6 \pm 1.4 \text{ g N ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $20.6 \pm 11.7 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow removal) in the first winter, and $56.1 \pm 40.2 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $18.4 \pm 7.9 \text{ g N ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $14.7 \pm 10.4 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow removal) in the second winter. The second peak of N₂O fluxes occurred in early May, with fluxes reaching $6.1 \pm 5.4 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $9.3 \pm 7.6 \text{ g N ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $291.4 \pm 136.5 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow removal) in the first winter, and $15.2 \pm 11.0 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $1087.9 \pm 318.5 \text{ g N ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $45.8 \pm 31.9 \text{ g N ha}^{-1} \text{ d}^{-1}$ (snow removal) in the second winter (Fig. 3a, b).

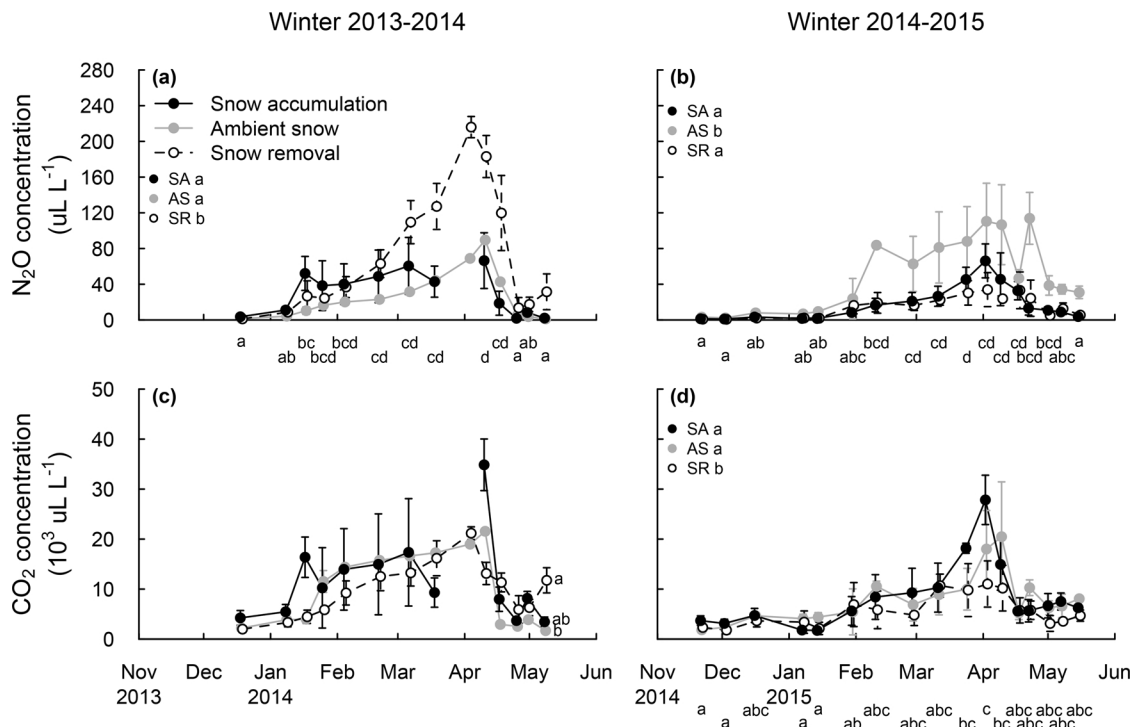


Fig. 3. Concentrations of N_2O (a, b) and CO_2 (c, d) at 15 cm soil depth in the first (a, c) and second (b, d) winters. Values are means ($n = 3$ or 4), and error bars are standard errors. Statistically significant differences are represented in three ways based on Tukey's test ($p < 0.05$): (1) when the sampling date by treatment interaction is significant, within-sampling date pairwise differences among treatments are indicated by lowercase letters next to mean points; and if the interaction is not significant, (2) differences among treatments are indicated with lowercase letters next to treatment abbreviations in the figure legend and (3) differences among sampling dates are indicated with lowercase letters aligned with sampling dates below the plot. SA: snow accumulation; AS: ambient snow; SR: snow removal.

In the first winter, spring thaw N_2O fluxes occurred earlier in snow removal plots than in other treatments (Fig. 4a). Sampling date and the sampling date by treatment interaction were significant, and emissions from snow removal plots were significantly higher than from other treatments on the last sampling date ($p < 0.001$), with a similar trend on several dates in April. In the second winter, spring thaw fluxes began earliest in snow removal plots, followed by snow accumulation plots and then ambient snow plots (Fig. 4b). Sampling date, treatment, and their interaction were all significant ($p < 0.0001$). Emissions from ambient plots were significantly higher than from snow removal plots on May 7 and May 15.

Soil or snow surface CO_2 fluxes varied significantly by sampling date for both winters, and followed a similar seasonal pattern to N_2O fluxes (Fig. 4c, d). Greatest fluxes were measured in either early winter or during spring thaw. In November through January, CO_2 fluxes ranged from 0.5 to $54.2 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in snow accumulation plots, 0.9 – $13.1 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in ambient snow plots and 0.01 – $5.8 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in snow removal plots during the first winter (treatment means by sampling date). In the second winter, CO_2 fluxes between November and January ranged from 0.2 to $2.2 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in snow accumulation plots, 0.1 – $8.0 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in ambient snow plots, and 0.3 – $3.5 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ in snow removal plots. During spring thaw (April and May), peak CO_2 fluxes were $6.9 \pm 4.7 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $3.8 \pm 1.2 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $28.3 \pm 10.8 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (snow removal) in the first winter, and $6.7 \pm 2.5 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (snow accumulation), $80.8 \pm 26.7 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (ambient snow) and $16.5 \pm 7.5 \times 10^3 \text{ g C ha}^{-1} \text{ d}^{-1}$ (snow removal) in the second winter ($p < 0.05$; Fig. 4). Pearson's correlations between CO_2 and N_2O gas fluxes or soil gas accumulation and winter conditions (snow depth, soil temperature, VWC) or analytes (NO_3^- , NH_4^+ and EOC) did not reveal any significant and meaningful linear relationships.

Overall, in the first winter, cumulative N_2O fluxes had a significant treatment-period interaction, with greater fluxes from snow removal

plots during spring thaw than several other treatment-period combinations (Table 1). Cumulative CO_2 fluxes were greater from snow accumulation plots during winter than for several other treatment-period combinations. In the second winter, cumulative N_2O and CO_2 fluxes were greater during spring thaw than winter. Fluxes from ambient snow plots were greater than both other treatments for N_2O , and greater than snow accumulation plots for CO_2 .

3.4. Denitrification, N_2O and CO_2 fluxes from laboratory assays

In the first winter, N_2O emission rates from soils incubated in the laboratory at field temperature were significantly greater in February and March than other dates. N_2O flux rates reached $43.9 \pm 9.5 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ in snow accumulation, $41.6 \pm 22.1 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ in ambient snow and $16.8 \pm 11.9 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ in snow removal plots in February and March (treatment means by sampling date) (Fig. 5a). Fluxes were significantly greater for soils from snow accumulation plots than snow removal plots, and intermediate in ambient snow plots. Denitrification followed a similar temporal pattern to N_2O emissions, and treatment effects were the same as for N_2O emissions. In February and March, peak denitrification rates were $34.4 \pm 6.2 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ in soils from snow removal plots, $30.8 \pm 18.9 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ from snow accumulation plots and $12.1 \pm 7.1 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ from snow removal plots (treatment means by sampling date) (Fig. 5b). Denitrification rates were comparable to N_2O emission rates, indicating minimal N_2 production. For CO_2 emissions, the sampling date by treatment interaction was significant, and emissions rates from soils from snow removal plots were lower than those from snow accumulation or ambient plots on several dates (Fig. 5c). In the second winter, N_2O emission rates had a significant sampling date by treatment interaction (Fig. 5d), with highest rates from ambient snow and sometimes snow accumulation soils on several winter dates. N_2O emission rates reached $11.1 \pm 6.1 \mu\text{g N kg}^{-1} \text{ dry soil h}^{-1}$ in snow accumulation,

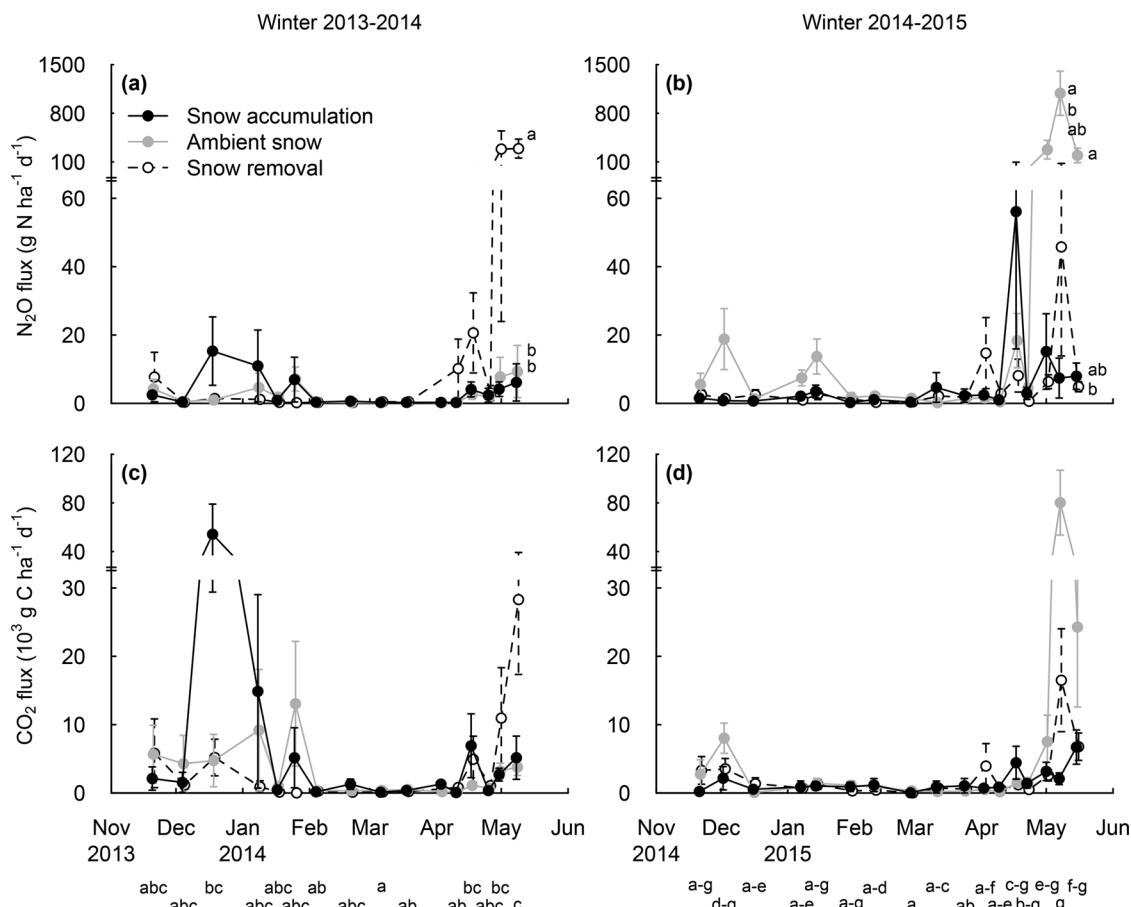


Fig. 4. Fluxes of N_2O (a, b) and CO_2 (c, d) over the soil or snow surface in the first (a, c) and second (b, d) winters. Values are means ($n = 3$ or 4), and error bars are standard errors. Statistically significant differences are represented in three ways based on Tukey's test ($p < 0.05$): (1) when the sampling date by treatment interaction is significant, within-sampling date pairwise differences among treatments are indicated by lowercase letters next to mean points; and if the interaction is not significant, (2) differences among treatments are indicated with lowercase letters next to treatment abbreviations in the figure legend and (3) differences among sampling dates are indicated with lowercase letters aligned with sampling dates below the plot. SA: snow accumulation; AS: ambient snow; SR: snow removal.

$26.3 \pm 9.1 \mu\text{g N kg}^{-1}$ dry soil h^{-1} in ambient snow and $1.0 \pm 0.4 \mu\text{g N kg}^{-1}$ dry soil h^{-1} in snow removal soils in February and March (treatment means by sampling date). Total denitrification also had a significant sampling date by treatment interaction, with no significant differences in pairwise comparisons within sampling dates. In February and March, the greatest denitrification rates were $13.0 \pm 6.7 \mu\text{g N kg}^{-1}$ dry soil h^{-1} for snow accumulation plots, $17.2 \pm 10.3 \mu\text{g N kg}^{-1}$ dry soil h^{-1} for ambient snow plots and $2.3 \pm 2.0 \mu\text{g N kg}^{-1}$ dry soil h^{-1} for snow removal plots (treatment

means by sampling date) (Fig. 5e). As with the first winter, denitrification rates were comparable to N_2O emissions, indicating minimal N_2 production. CO_2 emission rates had a significant date by treatment interaction, with lowest emissions from soils from snow removal plots on several dates in February and March (Fig. 5f).

3.5. Potential nitrification

In the first winter, potential nitrification rates had a significant

Table 1

Cumulative in situ gaseous emissions in winter and spring thaw periods for both winters (N_2O : g N ha^{-1} winter $^{-1}$; CO_2 : 10^3 g C ha^{-1} winter $^{-1}$). Values are means \pm s.e. across replicate plots. Statistics are from two-way ANOVAs conducted across treatment and period, within winter. The distinction between seasonal periods is defined based on soil frost as described in the materials and methods section.

Gas	Treatment	Winter 2013–2014 ¹		Winter 2014–2015	
		Winter	Spring thaw	Winter ^{a,2}	Spring thaw ^b
N_2O	Snow accumulation	$689 \pm 256^{\text{ab}}$	$126 \pm 44^{\text{ab}}$	233 ± 124^3	805 ± 389
	Ambient snow	$244 \pm 81^{\text{ab}}$	$150 \pm 86^{\text{a}}$	780 ± 150	13389 ± 2688
	Snow removal	$144 \pm 70^{\text{a}}$	$4338 \pm 2719^{\text{b}}$	221 ± 38	712 ± 235
CO_2	Snow accumulation	$1719 \pm 560^{\text{a}}$	$145 \pm 63^{\text{b}}$	151 ± 69^4	161 ± 39
	Ambient snow	$588 \pm 266^{\text{ab}}$	$69 \pm 7^{\text{b}}$	231 ± 30	263 ± 68
	Snow removal	$221 \pm 94^{\text{b}}$	$319 \pm 115^{\text{ab}}$	176 ± 47	941 ± 218

¹ For winter 2013–2014, different lowercase letters indicate statistically significant pairwise differences in the case of significant treatment-period interactions.

² Lowercase letters indicate that spring thaw fluxes were significantly greater than winter fluxes for both gases.

³ Fluxes were significantly greater for the ambient snow treatment than other treatments.

⁴ Fluxes were significantly greater for the ambient snow treatment than the snow accumulation treatment.

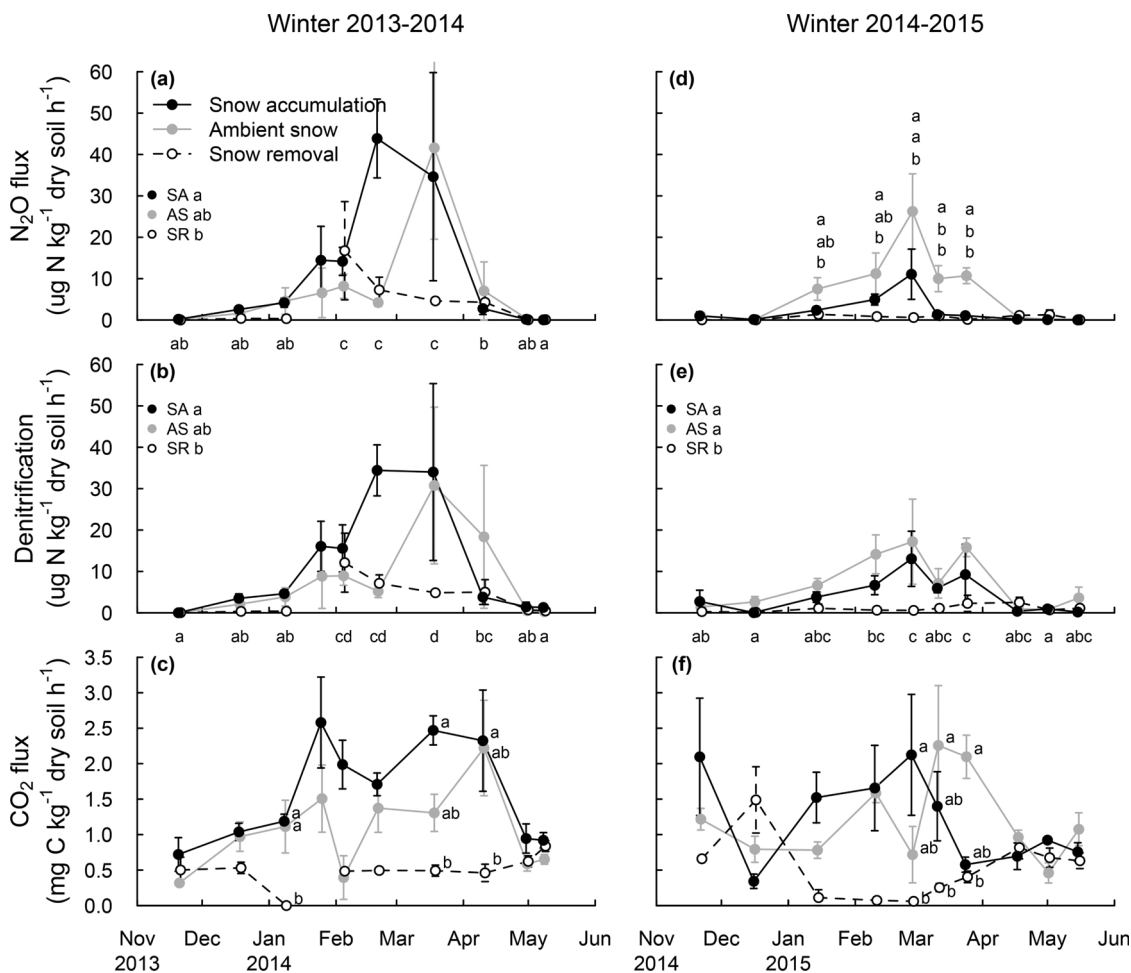


Fig. 5. N₂O flux (a, d), total denitrification, measured as N₂O flux under acetylene treatment (b, e), and CO₂ flux measured using laboratory assays. Soils were incubated at in situ temperatures, in the first (a, c) and second (b, d) winters. Values are means (n = 3), and error bars are standard errors. Statistically significant differences are represented in three ways based on Tukey's test ($p < 0.05$): (1) when the sampling date by treatment interaction is significant, within-sampling date pairwise differences among treatments are indicated by lowercase letters next to mean points; and if the interaction is not significant, (2) differences among treatments are indicated with lowercase letters next to treatment abbreviations in the figure legend and (3) differences among sampling dates are indicated with lowercase letters aligned with sampling dates below the plot. SA: snow accumulation; AS: ambient snow; SR: snow removal.

sampling date by treatment interaction (Fig. 6a). Despite this significant interaction, there were no significant differences among treatments in pairwise comparisons within each sampling date. In the second winter, potential nitrification varied significantly by date, with increasing rates from November to January, a decrease to lowest rates in March, and an increase over spring thaw (Fig. 6b).

3.6. Soil analytes and soil moisture

In the first winter, soil ammonium concentrations were significantly higher in snow removal and snow addition plots than in ambient snow plots. Soil ammonium concentrations peaked in March and April and decreased during spring thaw (Fig. 7a, $p < 0.001$). Soil nitrate

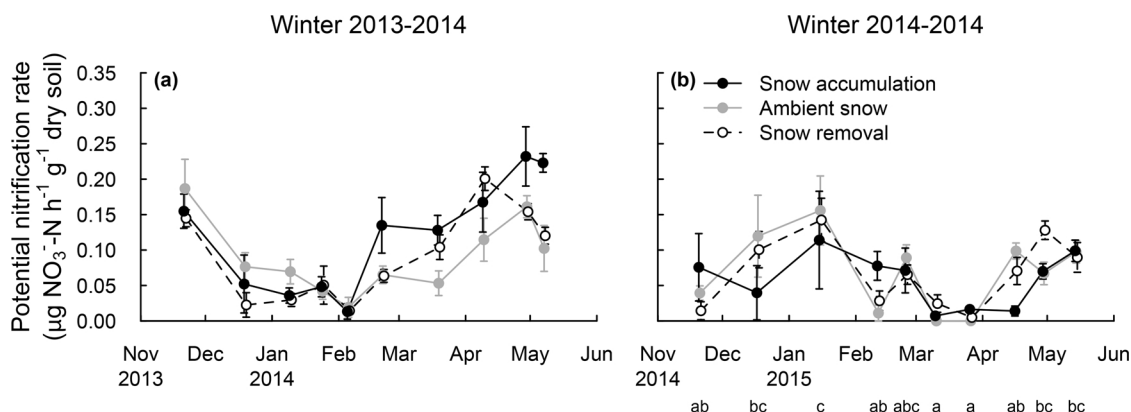


Fig. 6. Potential nitrification rates, measured as production of NO₃⁻ over time at room temperature, in the first (a) and second (b) winters. Values are means across replicates (n = 3), and error bars are standard errors. As there were no significant sampling date by treatment interactions, statistically significant differences among sampling dates are indicated with lowercase letters aligned with sampling dates below the plot.

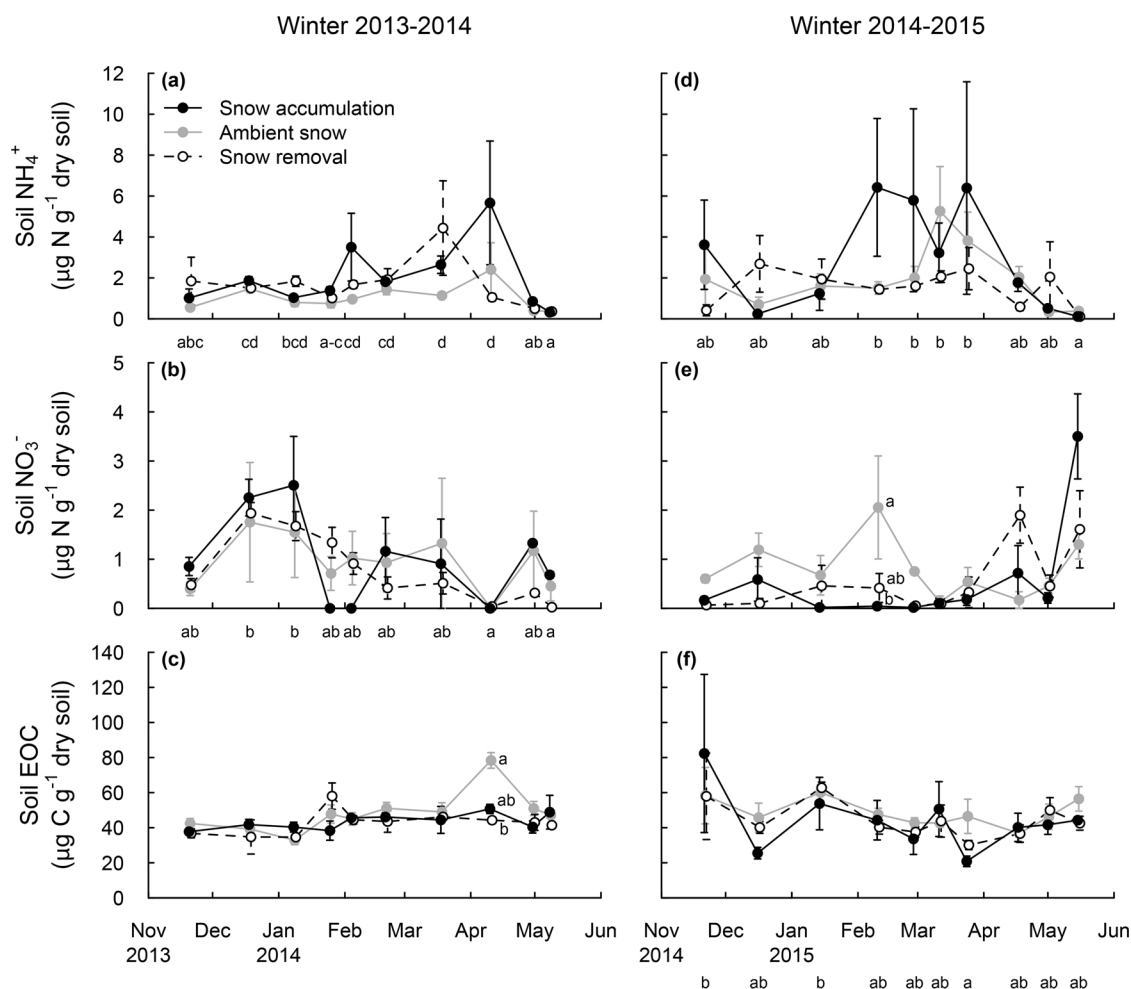


Fig. 7. Soil $\text{NH}_4^+\text{-N}$ (a, d), $\text{NO}_3^-\text{-N}$ (b, e), and extractable organic carbon (EOC) (c, f) in the first (a) and second (b) winters. Values are means across replicates ($n = 3$), and error bars are standard errors. Statistically significant differences are represented in three ways based on Tukey's test ($p < 0.05$): (1) when the sampling date by treatment interaction is significant, within-sampling date pairwise differences among treatments are indicated by lowercase letters next to mean points; and if the interaction is not significant, (2) differences among treatments are indicated with lowercase letters next to treatment abbreviations in the figure legend and (3) differences between sampling dates are indicated with lowercase letters aligned with sampling dates below the plot. SA: snow accumulation; AS: ambient snow; SR: snow removal.

Table 2

Gravimetric water content of collected experimental soil (including any soil ice) in winter and spring thaw periods for both winters (g water g^{-1} dry soil). Values are means \pm s.e. across replicate plots. Statistically significant differences, as denoted by lowercase letters, are from two-way ANOVAs conducted across treatment, period, and sampling year; all main effects were significant, and interactions were not. The distinction between seasonal periods is defined based on soil frost as described in the material and method section.

	Winter 2013–2014 ^a		Winter 2014–2015 ^b	
	Winter ^a	Thaw ^b	Winter ^a	Thaw ^b
Snow accumulation ^a	0.66 \pm 0.04	0.52 \pm 0.11	0.49 \pm 0.02	0.26 \pm 0.02
Ambient snow ^a	0.80 \pm 0.06	0.57 \pm 0.13	0.56 \pm 0.02	0.30 \pm 0.02
Snow removal ^b	0.55 \pm 0.05	0.32 \pm 0.02	0.38 \pm 0.01	0.27 \pm 0.02

concentrations also varied with sampling dates, with highest concentrations in December and early January (Fig. 7b). Higher nitrate concentrations typically occurred when ammonium concentrations were relatively low, and vice versa, but there was no significant correlation between nitrate and ammonium concentrations. Extractable organic carbon concentrations had a significant date by treatment interaction, and were highest in ambient snow soils in mid-April (Fig. 7c).

In the second winter, soil ammonium concentrations varied by sampling date, with highest concentrations in February and March (Fig. 7d). Soil nitrate concentrations had a sampling date by treatment interaction, with variation among treatments on one sampling date

(Fig. 7e). Nitrate and ammonium concentrations were inversely correlated ($p = 0.01$). Extractable organic carbon concentrations varied with sampling date, but concentrations were relatively consistent throughout the winter, with only a few dates with higher or lower concentrations (Fig. 7f).

Gravimetric water content of collected experimental soils (GWC) was significantly greater in the first winter than the second winter, significantly greater mid-winter than during thaw, and significantly lower in snow removal plots than in snow accumulation and ambient snow plots (Table 2).

4. Discussion

4.1. Effects of snow manipulation treatments on greenhouse gas emissions

Snow manipulation treatments significantly affected N_2O accumulation and fluxes in both study winters, but treatment effects varied by winter. In the first winter, the snow removal treatment was associated with greatest soil gas accumulation and spring thaw fluxes, whereas in the second year, greatest accumulation and fluxes were associated with the ambient snow treatment (Figs. 3 and 4). Snow removal studies on agricultural soils in Japan and Finland have found similar effects to our first winter (Maljanen et al., 2007, 2009; Yanai et al., 2011). The authors attributed this to increased soil C and N availability with soil frost; however, effects varied across soil types (Maljanen et al., 2007, 2009; Yanai et al., 2011). Freeze-thaw events and snowmelt have been associated with declines in soil microbial biomass C and N and increased mineralization in many systems, although responses have varied, likely due to differences in soil types or to treatment severity compared to typical in situ conditions (Brooks et al., 1998; Larsen et al., 2002; Kumar et al., 2013). This mechanism could have contributed to high fluxes from snow removal plots in the first winter in our study. Alternatively, variation in N_2O accumulation and fluxes could have been related to gas diffusivity into soils. In the first winter, snow-covered plots had substantial ice in and on the soil, whereas snow removal plots had lower surface ice coverage and drier soil (collected soil sample gravimetric water content). This relatively drier soil could have resulted in greater gas diffusivity and soil O_2 availability, allowing for greater nitrification and availability of NO_3^- for denitrification. In contrast, basal ice layers in snow-covered plots likely reduced gas diffusivity, potentially facilitating complete reduction of N_2O to N_2 and inhibition of nitrification via decreased O_2 diffusion, limiting NO_3^- supply to denitrifiers. This is supported by low rates of potential nitrification in early and mid-winter, indicating that in situ soil conditions were not optimal for nitrifier growth and enzyme production. In addition, a rough calculation of the amount of time for which bulk soil NO_3^- could support measured mid-winter denitrification rates suggests that NO_3^- could become limiting in areas of the soil with greater activity, especially as substrate diffusion is decreased in frozen soils.

Greater N_2O accumulation and fluxes from ambient snow soils compared to other treatments in the second winter could be explained by an increase in disruption of organic matter due to the greater soil water content during the extended period of freeze-thaw cycles before snow cover stabilized soil temperatures in February (Fig. 2d–f). Bullock et al. (1988) showed that disruption of soil aggregates with freezing increased with soil water content in both field and laboratory studies, demonstrating a link between soil water content and substrate release (Christensen and Christensen, 1991). Whether water content or freezing temperature has a greater effect on substrate release and N_2O emissions is uncertain, however, and likely depends on the relative degree of each factor (Koponen and Martikainen, 2004; Teepe et al., 2004).

In the second winter, greater sand content of the site, bare soils during the mid-January thaw (due to complete snow melting), and the consequent lack of basal ice layer formation may have contributed to better drainage throughout the winter by lowering soil water content and facilitating greater gas diffusivity than in the previous winter (Table 2). Ambient snow plots had greatest soil volumetric water content both before freezing in December and during spring thaw in April, which may have increased the presence of anoxic microsites relative to other treatments and facilitated substantial N_2O accumulation and fluxes (Fig. 2f). In contrast, drier soil and greater mid-winter O_2 availability in snow removal and snow accumulation plots may have limited N_2O production relative to ambient snow plots. Other studies have also found relationships between water content or water-filled pore space and N_2O emissions in frozen soils (Teepe et al., 2000; Koponen and Martikainen, 2004), or interpreted spatial and inter-annual variation in situ fluxes to be related to soil water content (van

Bochove et al., 2000). In a similar vein, sandy frozen soils have less unfrozen water than clayey soils at a given sub-zero temperature, and have been associated with decreased nitrification attributed to limitation of substrate (NH_4^+) diffusion (Clark et al., 2009). Hypothetically, decreased nitrification could decrease N_2O production through limitation by NO_3^- , linking unfrozen water content with N_2O production. In ambient snow soils, greater water content, associated with more unfrozen water at freezing temperatures (Brooks et al., 2011), could have contributed to N_2O production through increased solute diffusivity (NO_3^- and NH_4^+).

Our hypotheses for explaining treatment effects on N_2O dynamics over both winters essentially fall into two categories: 1) mechanisms acting via carbon substrates, and 2) mechanisms acting via gas diffusivity, O_2 availability, and N substrates (NO_3^-). Another piece of evidence may come from the relationship between N_2O and CO_2 concentrations in soil throughout the winter. Although soil N_2O and CO_2 concentrations were positively correlated, indicating the expected relationship between denitrification and respiration, the slope of this relationship depended on treatment. Snow removal plots in the first winter, and ambient snow plots in the second winter, had a relatively greater ratio of N_2O to CO_2 throughout the winter (ANCOVA results). This means that with any change in CO_2 concentration, N_2O concentration changed more quickly in soils in these plots. This suggests that N_2O and CO_2 production responded differently to snow manipulation treatments. This enhanced N_2O production may implicate factors that facilitate denitrification, such as soil gas diffusivity or NO_3^- availability, rather than those that also facilitate aerobic respiration, i.e. C availability or direct temperature effects on metabolic rates. Alternatively, greater effects of freezing or freeze-thaw on N_2O than CO_2 production could be related to C availability, if a substrate increase is minimal in the context of high aerobic respiration, but essential for denitrifiers competing for this organic C (Koponen and Martikainen, 2004).

4.2. Relationship between winter conditions and spring fluxes

Treatments with the greatest mid-winter soil N_2O accumulation also had the greatest spring thaw N_2O fluxes, particularly when soils were above 0°C and fluxes were unlikely to be due to passive degassing. This could be due to consistent treatment differences in soil conditions. For example, in the second winter, greater soil water content before and after freezing was observed in ambient snow plots but not in other treatments (Fig. 2f). However, in the first year, spring soil temperature was comparable across treatments (Fig. 2b), and gravimetric water content in collected samples was comparable by late April (data not shown). Persistent differences in N_2O dynamics could also be due to a legacy effect: a connection between winter soil conditions and spring fluxes could, for example, be related to the abundance of soil denitrification enzymes. Legacy effects have been demonstrated in a laboratory experiment linking N_2O emissions and microbial activity and composition to previous moisture conditions in unfrozen soils (Banerjee et al., 2015). Alternatively, mid-winter soil N_2O gas accumulation and spring thaw fluxes might be due to different mechanisms. For example, greater cumulative spring thaw CO_2 fluxes in ambient plots in the second winter compared to other treatments, but comparable mid-winter soil CO_2 as compared to snow accumulation plots, may indicate the role of organic C in stimulating N_2O fluxes in spring but not winter (Fig. 4b, d). This could have occurred despite comparable soil EOC (Fig. 7) if quick turnover of mineralized C prevented notable changes in pool size.

CO_2 and N_2O surface fluxes and soil gas accumulation were not significantly linearly correlated with winter soil conditions (snow depth, soil temperature, VWC) or soil analytes (NO_3^- , NH_4^+ and EOC). This temporal mismatch between gas dynamics and soil factors was likely due to a combination of factors. First, ice lenses and frozen soil affected the timing of winter surface fluxes and soil gas accumulation.

For surface fluxes, decreased diffusion to the surface meant that the timing of maximum gas production (and associated environmental conditions) did not necessarily match the timing of maximum surface gas release. For soil gas concentrations, weeks of accumulation meant that the timing of maximum concentration did not indicate the timing of maximum production. Second, greatest fluxes occurred at thaw when temperatures were about 0 °C and moderate VWC was observed, indicating non-linear relationships between surface fluxes and soil temperature and water content.

4.3. Dynamics of soil gas accumulation and emissions over time

In both winters, soil N₂O and CO₂ concentrations at 15 cm depth began to increase in mid- to late January, accumulated while soils were frozen and surface fluxes were negligible, and reached greatest concentrations in early April at the start of spring thaw (Figs. 3 and 4). This pattern has been observed in other agricultural systems, and has been similarly attributed to restricted gas diffusion due to ice lenses in the soil (Cates and Keeney, 1987; Burton and Beauchamp, 1994; Risk et al., 2014) and in the snowpack, along with basal ice layers on the soil surface (van Bochove et al., 2001; Maljanen et al., 2007; Risk et al., 2014). van Bochove et al. (2001) suggested that basal ice layers may become more common with climate change-related increases in winter rain events, such as was observed in the first winter. However, relative effects of basal ice layers on soil gases are uncertain. Maximum soil N₂O concentrations for snow-covered plots in the first winter, which had basal ice layers, were less than those for snow-removal plots, for which surface ice was spatially and temporally discontinuous, but comparable to or greater than soil N₂O concentrations in the second winter, when there were no basal ice layers (Fig. 3).

Early winter emissions from snow-covered plots support evidence that N₂O emissions from agricultural soils can continue under snow throughout the winter (Röver et al., 1998; Teepe et al., 2000; van Bochove et al., 2000). This is likely due to denitrification in unfrozen films of water in the surface soil and/or the escape of gases produced in deeper soil through cracks in frost layers (Teepe et al., 2001), and may indicate that soil temperature stabilization facilitated heterotrophic microbial activity (Brooks et al., 2011; Blankinship and Hart, 2012). These results highlight the importance of measuring soil gas accumulation to assess mid-winter N₂O production, since there were low to negligible emissions when ice lenses in the soil and snowpack were present.

In both winters, N₂O fluxes had an initial peak in mid-April that coincided with a decrease in soil gas concentration, followed by a second peak in early May that coincided with low to negligible soil N₂O (Figs. 3 and 4). A double peak pattern has been observed during spring thaw in other agricultural systems, with the first peak attributed to passive degassing and the second peak attributed to de novo production (Cates and Keeney, 1987; Teepe et al., 2001; Maljanen et al., 2009). In our study, passive degassing is further supported by relatively low mid-April denitrification rates in both winters (Fig. 5b, e) and, in the first winter, greatest mid-April fluxes from snow removal plots, which had greatest soil N₂O concentrations. In contrast, experiments using isotopically labeled inorganic N or soil N₂O mass calculations suggested that during spring thaw, N₂O deeper than ~12.5 cm was fully reduced to N₂, and emissions were due to de novo production and some degassing from the surface soil (van Groenigen et al., 2005; Wagner-Riddle et al., 2008; Risk et al., 2014).

4.4. Denitrification and N₂O emissions

Mid-winter denitrification rates (Fig. 5) corroborate previous reports of substantial denitrification in frozen soil in eastern Canada (Wertz et al., 2013; Tatti et al., 2014, 2015) and support measurements of sub-surface N₂O accumulation (Fig. 4). Temporal and treatment patterns for denitrification rates sometimes differed from those for in

situ gas flux measurements. For example, in the first winter, soil removal plots had greatest soil N₂O accumulation but lowest denitrification compared to other treatments (Figs. 3–5). This result could indicate rate differences between the very cold surface soil used for denitrification assays and the in situ N₂O emissions from deeper soil layers. Measurements with and without acetylene suggest that emissions were predominantly N₂O, with minimal reduction to N₂, possibly due to low-temperature limitation of N₂O reductase (Melin and Nömmik, 1983; Sahrawat and Keeney, 1986; Holtan-Hartwig et al., 2002). Similar results have been found for other frozen agricultural (Wagner-Riddle et al., 2010; Tatti et al., 2014) and forest (Öquist et al., 2004) soils. Limited N₂O reduction is unlikely to be an artifact of soil collection via increases in O₂ availability, nitrification, and NO₃[−] availability favoring the reduction of earlier intermediates (Focht et al., 1974; Dendooven et al., 1994), as greatest denitrification rates in early winter of the first year were concurrent with low nitrification potential (Figs. 5 and 6).

4.5. Climate change effects on winter N cycling

Results from in situ N₂O and soil N cycling process measurements from both winters of this study indicate that potential climate-related changes in snow depth are unlikely to have a straightforward effect on greenhouse gas production and emissions. Rather, effects are likely to be mediated by both soil water content and temperature, and may therefore work in concert with variation in soil texture and other factors that affect soil physical conditions. This is supported by variation in treatment effects between study winters, as well as the temporal discontinuity between soil N₂O accumulation and surface fluxes. Furthermore, the association between over-winter soil gas accumulation and the magnitude of spring thaw surface fluxes suggests the hypothesis that these fluxes are dependent on conditions not only during spring thaw, but also during the previous winter. Overall, these findings suggest that better predictions of winter and spring emissions will depend on a better understanding of the physical mechanisms that control N₂O production and release in frozen and thawing soils, in situ as well as in the laboratory.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.agee.2018.01.033>.

References

- Banerjee, S., Helgason, B., Wang, L., Winsley, T., Ferrari, B.C., Siciliano, S.D., 2015. Legacy effects of soil moisture on microbial community structure and N₂O emissions. *Soil Biol. Biochem.* 95, 40–50.
- Blankinship, J.C., Hart, S.C., 2012. Consequences of manipulated snow cover on soil gaseous emission and N retention in the growing season: a meta-analysis. *Ecosphere* 3, 1–20.
- Brooks, P.D., Williams, M.W., Schmidt, S.K., 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry* 43, 1–15.
- Brooks, P.D., Grogan, P., Templer, P.H., Groffman, P.M., Öquist, M.G., Schimel, J.P., 2011. Carbon and nitrogen cycling in snow-covered environments. *Geogr. Compass* 5, 682–699.
- Bullock, M.S., Nelson, S.D., Kemper, W.D., 1988. Soil cohesion as affected by freezing,

- water content, time and tillage. *Soil Sci. Soc. Am. J.* 52, 770.
- Burton, D.L., Beauchamp, E.G., 1994. Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. *Soil Sci. Soc. Am. J.* 58, 115–122.
- Burton, D.L., 2013. Constructing a Soil Atmosphere Probe. <https://www.dal.ca/faculty/agriculture/research/centres-and-labs/greenhouse-gas-lab.html>.
- Cates Jr., R.L., Keeney, D.R., 1987. Nitrous oxide production throughout the year from fertilized and manured maize fields. *J. Environ. Qual.* 16, 443–447.
- Chantigny, M.H., Rochette, P., Angers, D.A., Goyer, C., Brin, L.D., Bertrand, N., 2017. Nongrowing season N₂O and CO₂ emissions: temporal dynamics and influence of soil texture and fall-applied manure. *Can. J. Soil Sci.* 97, 452–464.
- Christensen, S., Christensen, B.T., 1991. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. *J. Soil Sci.* 42, 637–647.
- Christensen, S., Tiedje, J.M., 1990. Brief and vigorous N₂O production by soil at spring thaw. *J. Soil Sci.* 41, 1–4.
- Clark, K., Chantigny, M.H., Angers, D. a., Rochette, P., L-É, Parent, 2009. Nitrogen transformations in cold and frozen agricultural soils following organic amendments. *Soil Biol. Biochem.* 41, 348–356.
- Dörsch, P., Bakken, L.R., 2004. Low-temperature response of denitrification: comparison of soils. *Eurasian Soil Sci.* 37, S102–S106.
- Davidson, E.A., Kanter, D., 2014. Inventories and scenarios of nitrous oxide emissions. *Environ. Res. Lett.* 9, 105012.
- Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V., Veldkamp, E., 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. *Bioscience* 50, 667.
- Dendooven, L., Splatt, P., Anderson, J.M., Scholefield, D., 1994. Kinetics of the denitrification process in a soil under permanent pasture. *Soil Biol. Biochem.* 26, 361–370.
- Dorland, S., Beauchamp, E.G., 1991. Denitrification and ammonification at low soil temperatures. *Can. J. Soil Sci.* 71, 293–303.
- Environment Canada Canadian Climate Normals: 1981–2010. Climate Normals & Averages.**
- Firestone, M.K., Firestone, R.B., Tiedje, J.M., 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* 208, 749–751.
- Focht, D.D., Stolzy, L.H., Meek, B.D., 1974. Sequential reduction of nitrate and nitrous oxide under field conditions as brought about by organic amendments and irrigation management. *Soil Biol. Biochem.* 11, 37–46.
- Goodroad, L.L., Keeney, D.R., 1984. Nitrous oxide emissions from soils during thawing. *Can. J. Soil Sci.* 64, 187–194.
- Groffman, P.M., Holland, E.A., Myrold, D.D., Robertson, G.P., Zou, X., 1999. Denitrification. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), *Standard Soil Methods for Long Term Ecological Research*. Oxford University Press, New York, pp. 272–288.
- Hart, S.C., Stark, J.M., 1997. Nitrogen limitation of the microbial biomass in an old-growth forest soil. *Ecoscience* 4, 91–98.
- Hayhoe, K., Wake, C.P., Huntington, T.G., et al., 2006. Past and future changes in climate and hydrological indicators in the US Northeast. *Clim. Dyn.* 28, 381–407.
- Hofmann, A.F., Soetaert, K., Middelburg, J.J., Meysman, F.J.R., 2010. AquaEnv: an aquatic acid–base modelling environment in R. *Aquat. Geochem.* 16, 507–546.
- Holtan-Hartwig, L., Dörsch, P., Bakken, L.R., 2002. Low temperature control of soil denitrifying communities: kinetics of N₂O production and reduction. *Soil Biol. Biochem.* 34, 1797–1806.
- Huntington, T.G., Hodgkins, G.A., Keim, B.D., Dudley, R.W., 2004. Changes in the proportion of precipitation occurring as snow in New England (1949–2000). *J. Clim.* 17, 2626–2636.
- Jones, H.G., 1999. The ecology of snow-covered systems: a brief overview of nutrient cycling and life in the cold. *Hydrol. Process.* 13, 2135–2147.
- Koponen, H.T., Martikainen, P.J., 2004. Soil water content and freezing temperature affect freeze–thaw related N₂O production in organic soil. *Nutr. Cycl. Agroecosyst.* 69, 213–219.
- Kumar, N., Grogan, P., Chu, H., Christiansen, C.T., Walker, V.K., 2013. The effect of freeze-thaw conditions on arctic soil bacterial communities. *Biology* 2, 356–377.
- Larsen, K.S., Jonasson, S., Michelsen, A., 2002. Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Appl. Soil Ecol.* 21, 187–195.
- Lenth, R.V., 2016. Least-squares means: the R package lsmeans. *J. Stat. Softw.* 69, 1–33.
- Mørkved, P.T., Dörsch, P., Henriksen, T.M., Bakken, L.R., 2006. N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biol. Biochem.* 38, 3411–3420.
- Maljanen, M., Kohonen, A.-R., Virkajärvi, P., Martikainen, P.J., 2007. Fluxes and production of N₂O, CO₂ and CH₄ in boreal agricultural soil during winter as affected by snow cover. *Tellus* 59B, 853–859.
- Maljanen, M., Virkajärvi, P., Hytönen, J., Öquist, M.G., Sparrman, T., Martikainen, P.J., 2009. Nitrous oxide production in boreal soils with variable organic matter content at low temperature – snow manipulation experiment. *Biogeosciences* 6, 2461–2473.
- Melin, J., Nömmik, H., 1983. Denitrification measurements in intact soil cores. *Acta Agric. Scand.* 33, 145–151.
- Nyborg, M., Laidlaw, J.W., Solberg, E.D., Malhi, S.S., 1997. Denitrification and nitrous oxide emissions from a black chernozemic soil during spring thaw in Alberta. *Can. J. Soil Sci.* 77, 153–160.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. *Nlme: Linear and Nonlinear Mixed Effects Models*. *Nlme: Linear and Nonlinear Mixed Effects Models*.
- Öquist, M.G., Nilsson, M., Sörensson, F., Kasimir-Klemetsson Å, Persson, T., Weslien, P., Klemetsson, L., 2004. Nitrous oxide production in a forest soil at low temperatures – processes and environmental controls. *FEMS Microbiol. Ecol.* 49, 371–378.
- R Core Team, 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Röver, M., Heinemeyer, O., Kaiser, E.A., 1998. Microbial induced nitrous oxide emissions from an arable soil during winter. *Soil Biol. Biochem.* 30, 1859–1865.
- Risk, N., Wagner-Riddle, C., Furon, A., Warland, J., Blodau, C., 2014. Comparison of simultaneous soil profile N₂O concentration and surface N₂O flux measurements overwinter and at spring thaw in an agricultural soil. *Soil Sci. Soc. Am. J.* 78, 180–193.
- Roper, J.D., Burton, D.L., Madani, A., Stratton, G.W., 2013. A simple method for quantifying dissolved nitrous oxide in tile drainage water. *Can. J. Soil Sci.* 93, 59–64.
- Sahrawat, K.L., Keeney, D.R., 1986. Nitrous oxide emission from soils. In: In: Stewart, B.A. (Ed.), *Advances in Soil Science Volume 4*. Springer, pp. 103–148.
- Sharratt, B.S., Baker, D.G., Wall, D.B., Skaggs, R.H., Ruschy, D.L., 1992. Snow depth required for near steady-state soil temperatures. *Agric. For. Meteorol.* 57, 243–251.
- Tatti, E., Goyer, C., Chantigny, M.H., Wertz, S., Zebarth, B.J., Burton, D.L., Filion, M., 2014. Influences of over winter conditions on denitrification and nitrous oxide-producing microorganism abundance and structure in an agricultural soil amended with different nitrogen sources. *Agric. Ecosyst. Environ.* 183, 47–59.
- Tatti, E., Goyer, C., Burton, D.L., Wertz, S., Zebarth, B.J., Chantigny, M.H., Filion, M., 2015. Tillage management and seasonal effects on denitrifier community abundance, gene expression and structure over winter. *Microb. Ecol.* 70, 795–808.
- Teepe, R., Brumme, R., Beese, F., 2000. Nitrous oxide emissions from frozen soils under agricultural, fallow and forest land. *Soil Biol. Biochem.* 32, 1807–1810.
- Teepe, R., Brumme, R., Beese, F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biol. Biochem.* 33, 1269–1275.
- Teepe, R., Vor, a., Beese, F., Ludwig, B., 2004. Emissions of N₂O from soils during cycles of freezing and thawing and the effects of soil water, texture and duration of freezing. *Eur. J. Soil Sci.* 55, 357–365.
- van Bochove, E., Jones, H.G., Bertrand, N., Prévost, D., 2000. Winter fluxes of greenhouse gases from snow covered agricultural soil: intra-annual and interannual variations. *Glob. Biogeochem. Cycles* 14, 113–125.
- van Bochove, E., Thériault, G., Rochette, P., Jones, H.G., Pomeroy, J.W., 2001. Thick ice layers in snow and frozen soil affecting gas emissions from agricultural soils during winter. *J. Geophys. Res.* 106, 23061–23071.
- van Groenigen, J.W., Georgi, P.J., Van Kessel, C., Hummelink, E.W.J., Velthof, G.L., Zwart, K.B., 2005. Subsoil ¹⁵N-N₂O concentrations in a sandy soil profile after application of ¹⁵N-fertilizer. *Nutr. Cycl. Agroecosyst.* 72, 13–25.
- Wagner-Riddle, C., Hu, Q.C., van Bochove, E., Jayasundara, S., 2008. Linking nitrous oxide flux during spring thaw to nitrate denitrification in the soil profile. *Soil Sci. Soc. Am. J.* 72, 908.
- Wagner-Riddle, C., Rapai, J., Warland, J., Furon, A., 2010. Nitrous oxide fluxes related to soil freeze and thaw periods identified using heat pulse probes. *Can. J. Soil Sci.* 90, 409–418.
- Wertz, S., Leigh, A.K.K., Grayston, S.J., 2012. Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiol. Ecol.* 79, 142–154.
- Wertz, S., Goyer, C., Zebarth, B.J., Burton, D.L., Tatti, E., Chantigny, M.H., Filion, M., 2013. Effects of temperatures near the freezing point on N₂O emissions, denitrification and on the abundance and structure of nitrifying and denitrifying soil communities. *FEMS Microbiol. Ecol.* 83, 242–254.
- Wipf, S., Rixen, C., 2010. A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Res.* 29, 95–109.
- Yanai, Y., Hirota, T., Iwata, Y., Nemoto, M., Nagata, O., Koga, N., 2011. Accumulation of nitrous oxide and depletion of oxygen in seasonally frozen soils in northern Japan – snow cover manipulation experiments. *Soil Biol. Biochem.* 43, 1779–1786.
- Yanai, Y., Hirota, T., Iwata, Y., Nemoto, M., Koga, N., Nagata, O., Ohkubo, S., 2014. Snow cover manipulation in agricultural fields: as an option for mitigating greenhouse gas emissions. *Ecol. Res.* 29, 535–545.